# Protocol plasmid loss

We would like to study the loss of plasmid of a bacterium during a certain time.

1- We have to choose a strain of bacterium and to transform it with a chosen plasmid that is not too heavy.
2- Once bacteria are transformed, we start a culture in a medium with antibiotic during few hours.
3- We take our culture, and we make a dilution of it until we can find absorption around 0,9.
4- Once we have our right absorption, we have to proceed with a series of dilutions that we will drop (5 µL) on a petri dish with a medium with antibiotic. Thanks to these dilutions, we want to determine which dilution it is better to start from in order to obtain one bacterium per 5 µL.

To do so we have to do a reading of our petri dish the next day. We will retain the dilution that is interesting for us, but also the two dilutions that frame the first one.

5- Once we know the dilutions that we have to do, we will start them from a culture with still an absorption of 0,9. The culture will be diluted into a medium without antibiotic. The experiment is starting.
6- In order to follow the evolution of the frequency of bacteria without plasmid, we will drop 5 µL of our culture every 2 hours on a petri dish with a medium with antibiotic (control) and another petri dish with a medium without antibiotic. We can proceed this way during 24 hours and compare the amount of colonies into the two petri dish in order to determine a frequency of plasmid loss.